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THE WATER BALANCE OF TSETSE FLIES

By E. BURSELL,*

(East African Trypanosomiasis Research Organisation, Tororo, Uganda)

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With 5 Text-figures

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I. A COMPARATIVE STUDY OF WATER BALANCE IN FLIES AT REST

1 INTRODUCTION

SEVERAL attempts have been made to relate differences in the resistance to desiccation of certain species of tsetse fly to differences in their habitat requirements (Buxton and Lewis, 1934; Jackson, 1945; Geigy and Huber, 1952); the general impression gained is that specific differences, if they exist at all, are of doubtful significance. But only a few species have been studied and the techniques employed cannot be considered to have been altogether adequate. For this reason it was thought that a more extensive comparative study of water balance would be worth while.

* Now at: Department of Zoology, University College of Rhodesia and Nyasaland, Salisbury, Southern Rhodesia.

For such an investigation the methods used in preliminary work on spiracular regulation (Bursell, 1957*b*) proved unsuitable, chiefly because the activity of the flies could be neither controlled nor measured. An apparent difference in resistance to desiccation might under these circumstances reflect nothing but a specific difference in activity. That such specific differences in the general level of activity occur has already been demonstrated (Bursell, 1957*a*).

To get a valid estimate of the resistance to desiccation of different species it was therefore necessary to use indirect means: in the first place the rate of water loss of the different species in dry air was determined for a given level of activity; and secondly the total quantity of available water reserves was estimated. The ratio between these two quantities

$$\frac{\text{reserves mg.}}{\text{loss mg./hr.}}$$

gives the duration of life at that level of activity and under those conditions of relative humidity. Provided that conditions are such that death is caused by desiccation, this in turn will give an unequivocal measure of resistance to desiccation. It is necessary to include, in the total water reserves, the amount of metabolic water produced by oxidation of fat; hence the fat consumption at the particular level of activity had also to be determined.

Teneral flies (that is, flies which have not yet taken a blood meal) have been used for most of the experiments since in such flies excretion is negligible and water is lost almost exclusively by transpiration.

2. MATERIALS AND METHODS

The species of *Glossina* investigated are listed below, with the localities from which they were collected.

Group	Species	Place	Lat. south	Long. east
Fusca	<i>G. fuscipleuris</i> Austen	Masindi	2.5	31.0
	<i>G. brevipalpis</i> Newstead	Mkata	7.0	37.5
	<i>G. longipennis</i> Corti	Kiboko	2.0	37.5
Palpalis	<i>G. palpalis fuscipes</i> Newstead	Kuja River	1.0	34.5
Morsitans	<i>G. pallidipes</i> Austen	Shinyanga	3.5	33.0
	<i>G. morsitans</i> Westwood	Singida	5.0	35.0
	<i>G. swynnertoni</i> Austen	Shinyanga	3.5	33.0
	<i>G. austeni</i> Newstead	Kilifi	3.5	40.0

With most of the species pupae collected in the field were used as a source of experimental material; but with *G. fuscipleuris* and *G. brevipalpis* the mortality of such pupae was extremely high and material had to be bred in the laboratory from females collected in the field.

Flies were isolated immediately after emergence from the puparium and left at 25° C. and 40 per cent. R.H. for two hours to allow hardening of their cuticles. Each fly was weighed in a small container on a Mettler balance reading to 0.01 mg., and then placed in a cylindrical cage made of bolting silk (diameter 4 cm., height 4 cm.) suspended on a fine glass rod made by drawing out 3 mm. glass tubing to a fine capillary. Movements of the fly in its cage caused vibrations of the glass rod and these were recorded on a smoked drum. The arrangement

was such that the cage could be suspended through a small hole in the glass lid of a desiccation chamber, the humidity of which was controlled with calcium chloride or potassium hydroxide solutions.¹

After a period of about sixteen hours flies were removed from the cages, killed with potassium cyanide vapour and weighed. The rate of transpiration was calculated on the basis of recorded weight loss: the amount of non-fatty solids lost in the course of the experiment amounted to no more than 0.1 mg.; and this loss was in large part counterbalanced by the concurrent gain in weight which results from the oxidation of fat (on an average about 0.80 mg. fat was consumed giving 0.86 mg. of water (*see page 216*)). The nett error introduced by equating water loss and weight loss will thus be no more than about 0.04 mg., or less than 2 per cent. of the weight loss normally recorded. Since the standard errors of the estimates are of the order of 10 per cent. of the mean it has been thought legitimate to neglect this source of error.

From the kymographic records an estimate of the percentage of the total time spent in activity could be obtained. Against these values were plotted the corresponding transpiration rates and from the graph the rate of loss of weight at any given level of activity could be gauged. It was found that under the conditions of the experiments the activity of flies was manifested largely in walking and running, short flights from one side of the cage to the other forming a relatively inconspicuous component. Activity of this kind probably bears little relation to the activity of flies under natural conditions, and comparison between species has therefore been based on the rate of water loss of flies at rest; values were obtained by regressing transpiration on percentage activity and calculating the loss at zero activity from the regression formula.

For the determination of water and fat content flies were dried to constant weight in a vacuum oven at 85° C. Care had to be taken in transferring flies from the vacuum oven to the balance as the adsorption of water vapour from the atmosphere was found to be extremely rapid. The procedure finally adopted was to desiccate the flies in shallow partitioned trays made of aluminium sheeting; at the end of the desiccation period air was admitted into the oven through a calcium chloride trap and the tray was then rapidly transferred into a dish containing phosphorus pentoxide. From this dish flies could be removed singly with forceps inserted through a small hole in the lid, and access to water vapour was thus limited to the transfer from dish to weighing pan, the balance cabinet itself being maintained at humidities as low as possible by dishes of calcium chloride. After weighing the trays were transferred to shallow dishes containing chloroform which entered the trays through perforations in the floor. After 12 hours they were drained and transferred to dishes of clean chloroform, and this process was repeated a second time. The flies were then exposed in the vacuum oven at 85° C. for two hours, reweighed, and the amount of chloroform-extractable substances (referred to throughout as fat) determined by subtraction.

Attempts were made to get estimates of resting fat loss by a method analogous to that used for water; in this case loss had to be determined with reference to control flies killed at the beginning of the experimental period. It was found that the variability of fat content in such flies was considerable and

¹ I am indebted to Mr. E. Whiteside, from whose earlier work in this Department (unpublished) this method of measuring activity was copied.

the estimated values of fat consumption consequently subject to errors so large as to render them almost useless.

To get a reliable estimate of fat consumption it was therefore necessary to go by way of the oxygen consumption. This was measured in a standard Warburg apparatus; to prevent flies from coming into contact with the alkali, a cylinder of bolting silk was placed round the central well in which was supported a roll of filter paper soaked in potassium hydroxide. One fly was introduced into each flask and a record made of its position and orientation every 10 minutes. Readings were only accepted as representing resting rates if there had been no change in either during the preceding 10 minutes. It was found that oxygen consumption, like water loss, was proportional to residual dry weight and results will be expressed in terms of this function.

All experiments on water balance were carried out at a temperature of $25.0 \pm 0.8^\circ \text{C}$.

3. RESULTS

(a) *The relation between size and transpiration rate*

One of the most striking differences between species of tsetse fly is the difference in size, with the biggest members of the group (e.g. *G. brevipalpis*) about five times as heavy as the smallest (e.g. *G. austeni*). In view of this wide range in size it is of great importance for comparative purposes to try to obtain an expression for the rate of water loss which will be independent of size. It is general experience that the loss expressed as a function of live weight is inadequate from this point of view; smaller individuals lose relatively more than larger ones, the reason usually assigned being that they have a relatively greater surface. This phenomenon has been confirmed with tsetse flies under a wide variety of conditions. In living flies it is to some extent masked by the regulatory processes described in a previous paper (Bursell, 1957b); small flies, which lose proportionately more to begin with, will control their loss more stringently at a later stage because their water reserves become more seriously depleted. Thus if the duration of an experiment is prolonged the two effects may to some extent cancel out and the percentage loss appear to be independent of size. This, however, does not mean that live weight would provide a satisfactory basis for the comparison of resting rates of loss, for it will be shown that with resting flies the degree of spiracular closure is independent of water content (see p. 211). Account must therefore be taken of differences in size, or in other words, the water loss must be expressed in terms which render it independent of size. It would be desirable, in fact, to study the relation between size and loss in the resting fly; this is, however, impracticable in view of the large errors involved in the estimation of resting loss. But if the relation can be studied in flies in which spiracular regulation has been prevented it is reasonable to suppose that the results will be applicable to the resting fly. The easiest way to avoid active regulation is by using dead insects, and a series of experiments was carried out as follows.

Flies were killed with potassium cyanide and left for 10 hours in 90 per cent. R.H. to allow full relaxation of the spiracular muscles. They were then exposed to 0 per cent. R.H. on wire trays for 2-3 hours and the loss in weight recorded. This loss was found to be positively correlated with weight (correla-

tion coefficient $r = 0.4-0.5$), that is, the larger flies lost more water than the smaller flies. When the loss was expressed as a percentage of original weight there was a negative correlation with size ($r = -0.5$ to -0.6); this confirmed that fresh weight is an unsatisfactory unit for the expression of water loss, and that surface effects might be playing an important part.

In trying to find an alternative unit in terms of which water loss could be expressed it was evidently impracticable, in an animal of such complex structure, to look for one naturally related to transpiration, and an empirical approach had to be made instead. The simplest way is to derive some function of weight which varies with size in the same way as does water loss, so that the rate of loss expressed in terms of this unit would be the same whatever the size. The use of fresh weight as a measure of size in tsetse flies has certain drawbacks; for the weight changes from one hour to the next and unless the fly is weighed at a very narrowly defined point in the life cycle the measure has little meaning. Linear measurements do not suffer from this disadvantage, and by direct measurement of a number of bodily dimensions one (a function of thoracic surface area, see Bursell, *in press* (a)) was found which was relatively easy to measure and at the same time satisfied the primary requirement of a suitable unit (the coefficient of correlation between rate of loss per cm^2 and size was $+0.0771$ and not significantly different from zero).

The validity of this function was, however, called in question by results from two samples of *G. morsitans*. These emerged in the laboratory from pupae obtained in the Central Province of Tanganyika, one in October 1956 and one in February 1957; they showed widely different rates of resting loss although the composition of the two batches was practically identical (water content 76.40 ± 0.14 per cent. and 76.46 ± 0.20 per cent.; fat content 37.40 ± 0.81 per cent. and 37.62 ± 1.02 per cent.). Further analysis of the data showed that the thoracic surface of the October flies was 10 per cent. greater in relation to their weight than that of the February flies; and furthermore that if the rate of water loss was expressed in terms of residual dry weight there was no significant difference between the groups. Concurrent investigations have shown that the relation between linear dimensions in general and dry weight is markedly influenced by the temperature obtaining during pupal development (Bursell, *in press* (a)), hence the difference between the October and the February samples. But what is of importance in the present context is that linear dimensions may alter by as much as 10 per cent. without having the slightest effect on the rate of water loss; clearly such dimensions are entirely unsuitable as a means of expressing transpiration rates in the tsetse fly. This point is laboured here as it is standard practice, in studies of arthropod water balance, to express the rate of transpiration as a function of surface area measured for a range of sizes, on the assumption that water loss is more closely related to surface area than to weight (e.g. Wigglesworth, 1945; Lees, 1947; Edney, 1951; Bursell, 1955; Holdgate, 1956, etc.). It is seldom established that this is in fact so, but even if it were, for a single series of animals, the present findings suggest that this practice may have little to recommend it; an empirical function of weight, which has the advantage of convenience, might be a more suitable unit.

It was thus necessary to look for an alternative to fresh weight and thoracic surface as a measure of size. Residual dry weight (*i.e.* dry weight after chloroform extraction) has the advantage over fresh weight that it remains relatively

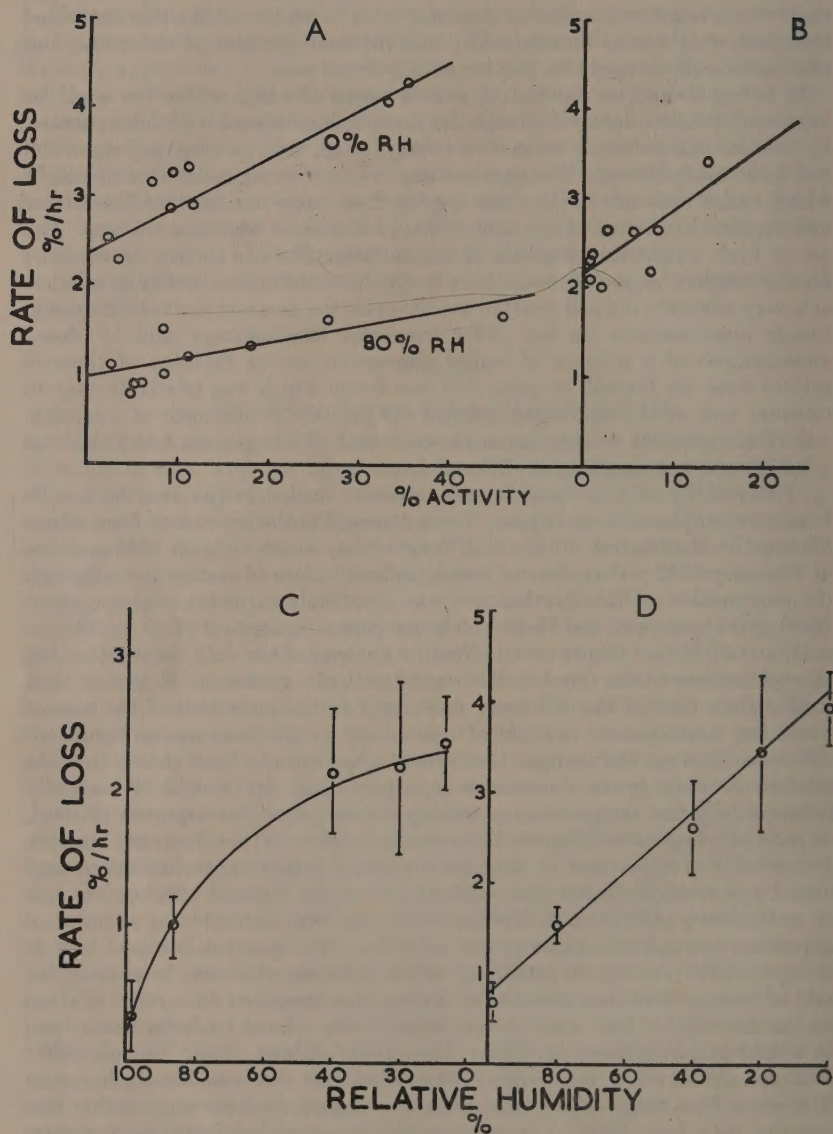


FIG. 1.—The transpiration of *G. morsitans*: (A) and (B) the effect of activity on the rate of loss of (A) tenerals at 0 per cent. and 80 per cent. R.H.; (B) non-tenerals at 0 per cent. R.H. (C) and (D) The effect of humidity on the (C) resting loss of tenerals; (D) loss of tenerals at 30 per cent. activity.

constant in the course of starvation; to determine whether it would satisfy the criterion previously laid down the water loss of 33 dead females and 33 dead males of *G. morsitans* in dry air was measured, and the residual dry weight of the flies subsequently determined. In both series there was a positive correlation between size and loss ($r_{\sigma\sigma} = +0.4108$ and $r_{\varphi\varphi} = +0.4050$), but when the loss was expressed as a percentage of the residual dry weight the rate of loss was found to be independent of size ($r_{\sigma\sigma} = -0.0597$; $r_{\varphi\varphi} = +0.0453$). This relation has been found to hold good in all species for which the number of flies available at any one time has been adequate to permit calculation of correlation coefficients.

It does not necessarily follow that because the rate of transpiration expressed as a percentage of residual dry weight is independent of size within a species, the use of this unit will also be capable of cancelling differences in size between species. But it is reasonable to suppose that its use will tend to that effect, and in what follows all rates of transpiration have been expressed as a percentage of residual dry weight.

(b) *The resting loss of G. morsitans*

(i) *The effect of humidity and water content.*

Figure 1A shows the effect of activity on the rate of weight loss of newly emerged *G. morsitans* males at 0 per cent. R.H. and at 80 per cent. R.H., and illustrates the kind of data from which estimates of resting loss are obtained. The resting loss at different humidities has been plotted in figure 1C; it shows the characteristic departure from the rectilinear familiar from earlier work (Bursell, 1957b). With the comparatively low levels of activity recorded in present circumstances it would not be legitimate to extrapolate to 100 per cent. activity and so determine whether a similar departure would characterise the active state as well. But consideration of the effect of humidity on the loss at 30 per cent. activity, when more than half of the total loss occurs during the active periods, suggests that this is not so, for at this level the relation between humidity and loss is adequately represented by a straight line (see fig. 1D). It would seem, therefore, that the effect of humidity on spiracular regulation demonstrated by the earlier experiments must be an effect shown only by the resting fly.

Some experiments were undertaken to find out whether water content, which is known to influence spiracular regulation (Bursell, 1957b), has any effect on the resting loss. A comparison of the relations between activity and water loss of teneral desiccated for one day at 20 per cent. R.H. with results already obtained for newly emerged tenerals showed no significant difference in the resting loss, but the slope of the regression line had decreased by about 40 per cent. As the significance of the difference could not be established with the data available, an experiment was done in which a simultaneous comparison was made of the two groups; the results are set out in Table I. From this it can be seen that water content has no effect on the resting loss of the flies, but that the loss at 30 per cent. activity is significantly lower in the desiccated group. The slope of the regression is again reduced but the difference does not reach the 5 per cent. level of significance.

TABLE I.—*The effect of desiccation for one day at 20 per cent. R.H. on the transpiration rate of G. morsitans males*

	Newly emerged	Desiccated	Diff.	S.E. diff.	P
<i>Experimental</i>					
Number	11	15			
Resting loss %/hr.	1.64	1.78	0.14	0.30	>0.9
Loss at 30% activity	4.30	3.55	0.75	0.32	0.05-0.02
<i>b</i>	0.089	0.059	0.029	0.022	0.2-0.1
Mean activity	9.4	12.7	3.3	3.3	0.4-0.3
<i>Controls, killed at the start</i>					
Number	13	13			
Water content %	76.02	74.13	1.89	0.29	<0.01
<i>Difference between experimentals and controls</i>					
Number	24	28			
Fat consumption mg.	0.536	0.540	0.006	0.211	>0.9

For estimates of fat consumption regressions were calculated in each case of fat content on size of flies, and the comparison made at the mean size for all groups.

b = regression coefficient of weight loss on activity.

S.E. diff. = the standard error of the difference.

There is a possibility that the recorded differences between the two groups may represent a difference in the kind of activity manifested, rather than a difference in regulatory adjustments; in other words that newly emerged teneral flies tend to spend a relatively greater proportion of their active periods in flight as opposed to walking. If this were so one would expect that newly emerged flies would show a relatively greater rate of fat consumption, the level of activity in the two groups being approximately equal. The fat loss was accordingly estimated with reference to control flies killed at the beginning of the experiment (see Table I); no difference could be established, and, although the evidence is by no means conclusive, the data may reasonably be taken as an indication that under conditions of activity the rate of transpiration is reduced in flies whose water content has been depleted, but that such depletion has no significant effect on the resting loss.

(ii) *Non-teneral flies.*

Figure 1B shows the rate of transpiration of male *G. morsitans* tested the day after their first feed. Excretion is normally copious in such flies, and loss by transpiration may constitute only a small proportion of total weight loss. For present purposes defaecation was prevented by anal block, a procedure which had no obvious deleterious effect on the flies during the experimental period. Comparison with figure 1A shows that the resting loss does not differ significantly from that of newly emerged flies, the calculated values being 2.15 ± 0.12 and 2.35 ± 0.17 per cent./hour; the slope of the regression is slightly less for the teneral flies but the difference is not statistically significant. These findings suggest that results obtained with teneral flies may with some confidence be applied to the non-teneral stages.

(iii) *Sex differences*

The resting loss of females of *G. morsitans* is lower than that of males, but the difference is slight and only in a test with more than 20 individuals of each sex did it reach the 5 per cent. level of significance (see p. 225, fig. 4). Two other species have been tested for sex difference (*G. swynnertoni* and *G. palpalis*) but in neither could a difference be established with the smaller numbers available.

(c) *The resting loss of other species*

The resting rates of water loss of some East African species of tsetse are set out in Table II. From these figures an impression is gained of very considerable homogeneity within the group. The lowest rates of loss differ from the highest by no more than a factor of 1.6 (cf. the corresponding difference factor in the pupa, which is more than 6 (see Bursell, 1958, Table VI, column 4)). Nor do the slight differences between species appear to bear any relation either to phylogeny or to habitat requirement; high and low values occur in the most primitive group (*G. longipennis* and *G. brevipalpis*) and in the most specialised group (*G. austeni* and *G. morsitans*); and the xerophilic *G. longipennis* shows higher rates of loss than any of the hygrophilic species (*G. fuscipleuris*, *G. brevipalpis*, *G. palpalis* and *G. austeni*). Only within the Morsitans Group does

TABLE II.—*The transpiration of different species of tsetse fly in dry air*

Group and species	N	Resting loss %/hr.	b	N	Dead loss %/hr.	Jackson's water loss %/hr.*
Fusca						
<i>G. fuscipleuris</i>	1	2.35	.	.	.	.
<i>G. brevipalpis</i>	6	2.54±0.12	0.093±0.036	.	.	.
<i>G. longipennis</i>	6	3.48±0.17	0.021±0.005	5	16.80±0.63	.
Palpalis						
<i>G. palpalis</i>	9	2.81±0.11	0.089±0.028	22	19.22±0.37	2.4
Morsitans						
<i>G. pallidipes</i>	10	2.60±0.13	0.075±0.015	10	21.90±0.47	.
<i>G. morsitans</i>	24	2.18±0.12	0.045±0.006	21	16.41±0.25	2.1
<i>G. swynnertoni</i>	7	2.68±0.16	0.062±0.005	10	23.65±0.68	2.6
<i>G. austeni</i>	7	3.26±0.20	0.036±0.074	11	25.48±0.95	.

The single specimen of *G. fuscipleuris* showed only 0.6 per cent. activity and its weight loss has been accepted as a resting rate.

b = the coefficient of regression of weight loss on per cent. activity.

N = the number of observations.

* Corrected for size.

there appear to be an indication of some correlation with habitat, in so far as *G. austeni* shows high rates compared with the savannah species. But in view of the opposite relation in the Fusca Group not much significance can be attached to this finding.

(d) *The water loss of dead flies*

Figures for the transpiration of dead flies are available for some of the species and they have been included in Table II. In general the values are about seven times greater than those for resting flies,² a difference which can be attributed to the opening of spiracles in dead flies (if the spiracles are blocked there is no difference between dead and living specimens).

But apart from this difference in general level there is broad agreement between the two sets of figures, at least in the *Palpalis* and *Morsitans* Groups—*G. morsitans* has the lowest value in both cases, *G. austeni* the highest and the other species are intermediate. The correlation breaks down for *G. longipennis*, whose resting loss is high in relation to the loss from dead flies. With this exception it would seem that the differences recorded in respect of resting loss reflect differences in the disposition, extent or waterproofing properties of the transpiring surfaces rather than differences in regulatory powers.

The lack of correlation between rates of transpiration and habitat requirement in the group as a whole suggests that changes in the nature of the tracheal system and improvements in the powers of spiracular regulation have played little part in the conquest of drier habitats. It would appear that the primitive tsetse flies were sufficiently well equipped in these respects to meet the demands made by such environments as have hitherto been invaded.

(e) *Water reserves*

The amount of water present in the newly emerged fly depends in part on the humidity to which the pupa has been subjected. To make valid inter-specific comparisons it is therefore necessary to ensure that pupae of different species are collected at a time when possible differences in soil humidity are at a minimum. It has been shown elsewhere (Bursell, 1958) that the humidity of pupal sites remains close to saturation for about a month after the end of the rains. Pupae of the savannah species which cannot be obtained in numbers during the rains were therefore collected as early as possible in the dry season. For species like *G. palpalis* and *G. austeni*, there is reason to suppose that the soil humidity never drops far below saturation.

Figure 2, line (a) shows the water content in milligrams of different species of tsetse fly, determined between one and two hours after emergence from the pupa. The data are plotted as a function of the residual dry weight; for each species the regression of water content on dry weight was determined and the standard errors of the estimated means calculated. These never exceeded 0.5 per cent. of the mean, and were in all cases too small to be represented graphically on the scale chosen.

There was no significant difference between species as regards the slope of the regression, and none differed significantly from the mean value which has been drawn as line (a) in figure 2. This line represents a water content of 76.2

² This difference is considerably greater than the 2-3-fold difference recorded for flies in air and in concentrations of CO₂ sufficient to cause opening of the spiracles (Bursell, 1957b). But the earlier experiments were carried out with flies in a state of partial activity, showing relatively high rates of loss in air (5.3 per cent./hr. as compared with resting values of 2.2 per cent./hr.), the activity having caused a relatively trivial increase in the transpiration of flies with open spiracles (20.3 per cent./hr. as compared with 16.4 per cent./hr.).

per cent. (of the non-fatty wet weight) and the value is constant for all species with the exception of *G. morsitans* with a water content of 77.0 per cent. This difference is a reflection of the fact that newly deposited pupae of *G. morsitans* have a higher water content than those of other species (see Bursell, 1958).

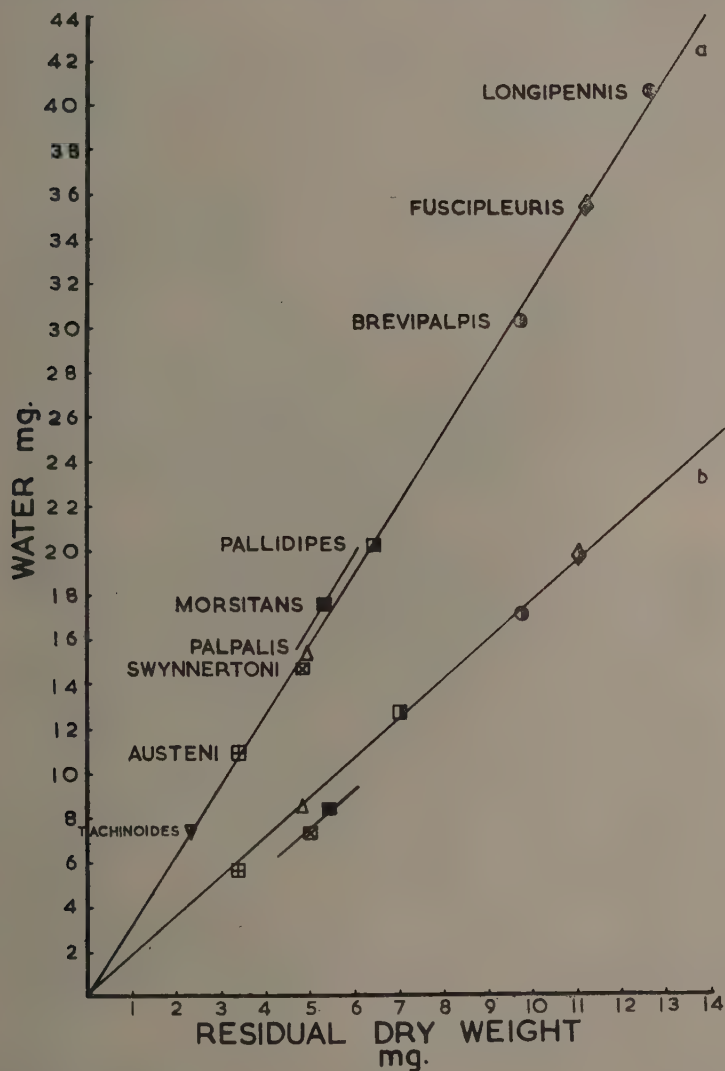


FIG. 2.—The water content of: (line *a*) newly emerged tsetse flies; (line *b*) flies starved to death in dry air.

The data for *G. fuscipleuris* and *G. brevipalpis* were obtained from pupae bred in the laboratory and hence subnormal in size. (The symbols used are the ones adopted at the Brazzaville conference of 1948.)

Figure 2 line (b) shows the water content of flies which could still respond by movements of the legs to mechanical stimulation, but which were incapable of performing the righting reaction. This has been taken to represent the critical water content, that is the lowest level to which water content may be reduced before death supervenes.

The data are plotted against original dry weight, as calculated from the mean size (thoracic surface) of the sample using a previously determined regression of dry weight on size with newly emerged teneral. This was done in order to allow for the slight decrease in residual dry weight which takes place in the course of starvation.

Here again the values for most of the species fall on a single line representing a water content of 64 per cent.³, from which none of the individual regressions departs significantly. A second line has to be drawn to accommodate *G. morsitans* and *G. swynnertoni* with a value of 60.6 per cent. This also is in accord with results obtained in connection with pupal water balance, which showed that the critical water content of pupae of these two species is lower than that of the others.

The water reserves of the different species of tsetse fly will then be represented by the difference between lines (a) and (b) at the appropriate level of residual dry weight. It is clear that, in proportion to size, this is greatest in *G. morsitans*, slightly lower in *G. swynnertoni* and still lower for all the other species examined, between which there is no significant difference.

(f) *Resting fat consumption and fat reserves*

Oxygen consumption has been determined only for *G. morsitans*; it was found to bear the same relation to size as does water loss, and it has been assumed that this relation holds good for all species.

The mean rate of oxygen consumption for teneral *G. morsitans* males at 25° C. was 5.240 ± 0.139 cmm./mg./hr. This is equivalent to about 20 cmm./100 mg. live weight/10 min., slightly lower than the values of resting oxygen consumption of *Calliphora erythrocephala* Meig. published by Thomson (1949).

Given that the iodine number of tsetse fat is 40–45 (A. R. Jenkins, *personal communication*) and assuming that the average chain length of fatty acids is 16 (see Baldwin, 1948), the combustion of 1 mg. of fat would require 2046 cmm. of oxygen, yielding 1.08 mg. of water. A resting oxygen consumption of 5.24 cmm./hr./mg. would therefore be equivalent to a fat consumption of 0.00256 mg./hr./mg. residual dry weight.

The production of metabolic water from fat will augment the existing water reserves of the resting fly by an amount corresponding to 1.08 times the resting fat consumption for the period of life, provided that the fat stores do not become exhausted before death by desiccation occurs. To determine whether this condition is fulfilled it was necessary to get an estimate of the extent of fat reserves in the different species.

The fat content of newly emerged flies depends in part on the temperature to which the pupae have been subjected during development (Bursell,

³ It must be emphasised that these values cannot be compared with values reported by other workers, since they represent water content as a percentage of original weight. The corresponding figures calculated as a percentage of residual dry weight at death are shown in Table III.

in press (b)). Since temperatures were not controlled for the samples of pupae under consideration the recorded differences do not give a very accurate picture of specific differences. But the range of temperatures covered under laboratory conditions in Shinyanga, at which even the pupae collected in the field had spent the latter half of their lives, does not exceed 4° C. and may for present purposes be ignored. With this reservation, the fat content of newly emerged teneral of the different species is given in Table III, column 3.

The fat reserves of a given species will be represented by the difference between these values and the lowest level to which fat content can be reduced before death occurs. This "critical fat content" was determined by starving flies to death at 80 per cent. R.H., water content being checked to ensure that death had not been caused by desiccation. Its value was found to be 4.2 per cent. of the total dry weight for the species studied in this respect (*G. pallidipes*, *G. morsitans* and *G. swynnertoni*) and it seems reasonable to suppose that the critical fat content may be taken as constant for the group as a whole.

Of the species included in Table III, the one whose fat reserves are most likely to be critically depleted during starvation in dry air is *G. swynnertoni*, which combines a small fat reserve with a high resistance to desiccation (see below). In fact, assuming a resting rate of fat consumption of 0.256 per cent./hr., the fat reserves of *G. swynnertoni* would last nearly 100 hours, which is well in excess of the estimated duration of life under conditions of desiccation. It may be concluded that in dry air fat reserves will not become exhausted in any of the species before death by desiccation supervenes.

(g) Resistance to desiccation

With the data available it is now possible to make valid comparisons between species in respect of resistance to desiccation. The relevant information is set out in Table III; column 4 shows the critical water content as a function

TABLE III.—The water and fat reserves of some species of tsetse fly

	Size (mg. RDW)	Fat content (% DW)	Critical water content (%)	Water reserves		
				Existing (mg.)	Metabolic (mg.)	Total (% RDW)
Fusca						
<i>G. brevipalpis</i>	11.8	.	65.7±0.39	16.4	1.7	153
<i>G. fuscipleuris</i>	11.8	26	65.6±0.23	16.4	2.1	157
<i>G. longipennis</i>	12.6	23	65.2±0.42	17.5	1.4	150
Palpalis						
<i>G. palpalis</i>	4.9	27	65.6±0.37	6.8	0.7	153
Morsitans						
<i>G. pallidipes</i>	6.7	35	66.1±0.33	9.3	1.0	154
<i>G. morsitans</i>	5.4	32	64.5±0.33	9.8	1.4	208
<i>G. swynnertoni</i>	4.9	23	64.0±0.41	8.2	0.9	186
<i>G. austeni</i>	3.4	32	65.4±0.37	4.7	0.5	153

RDW = residual dry weight = dry weight minus fat.

DW = dry weight.

Water content is expressed as a percentage of the non-fatty weight.

of the residual dry weight at death; column 2 the size of different species expressed in terms of residual dry weight; column 5 the quantity of expendable water reserves in milligrams, representing the difference between the upper and lower lines in figure 2. Expressing the reserves as a percentage of the residual dry weight and dividing by the resting rate of loss shown in Table II (column 3), it is possible to get an estimate of length of life in dry air if no metabolic water was produced; the metabolic water which would be produced during this period, assuming a resting rate of fat consumption of 0.256 per cent/hour, must be added to the existing water reserves and this gives a second estimate of longe-

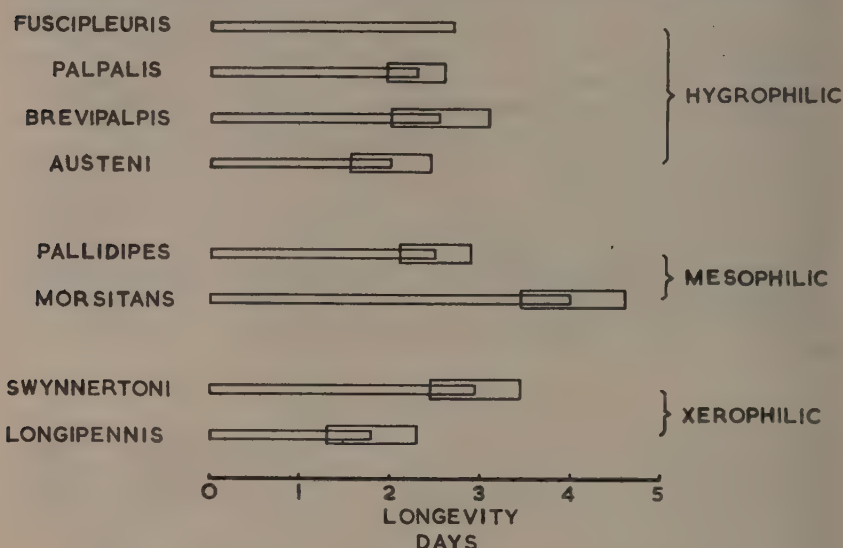


FIG. 3.—The longevity of different species of tsetse fly, at rest in dry air. (The approximate limits of error are shown by rectangles overlapping the mean.)

vity; the process is repeated so that estimates of total water reserves, expressed as a percentage of the residual dry weight in column 7, are arrived at by a series of successive approximations continued until a constant value was reached at the chosen level of accuracy. Dividing this figure by the value for the resting loss, the longevity in dry air is found.

These estimates of longevity will be subject to sampling errors both in respect of existing water reserves, metabolic water increment and resting rate of loss. Some idea of the magnitude of the over-all errors has been obtained by consideration of the 5 per cent. fiducial limits of the estimates of water reserves and of resting rate; the contribution made by metabolic water to the total reserves is slight and errors of estimates of this quantity have not been taken into consideration. The upper fiducial limit of water reserves has been divided by the lower fiducial limit of the resting rate and vice versa, to give approximate limits of longevity. The results are represented graphically in figure 3.

The species under consideration have been arranged in a sequence intended to represent increasing dryness of habitat. In drawing up this sequence I have

had the benefit of advice from a number of tsetse specialists in East Africa (see Acknowledgments). It was not possible to reach complete agreement on the order of arrangement and the sole responsibility for possible errors of interpretation must lie with the present writer. Some justification of the sequence will be put forward as it cannot pretend to the authority of a consensus of opinion.

G. fuscipleuris has been placed at the head of the series as an inhabitant of evergreen forest, in part overlapping with the true rain forest species like *G. fusca*, and limited to the high rainfall areas in western Uganda and near Lake Victoria. The distribution of *G. palpalis fuscipes* overlaps that of *G. fuscipleuris*, and this species also is limited to regions of high rainfall in the Western parts of Uganda and near the great lakes. For this reason it has been placed above *G. brevipalpis* which, although frequenting the evergreen vegetation of major drainage lines and coast bush, extends inland into areas of quite low rainfall. There is some indication that *G. austeni*, which is usually found associated with *G. brevipalpis*, has a preference for somewhat lighter thickets (Mr. R. J. B. Power, *personal communication*; and E.A.T.T.R.R.O., 1955) and it has accordingly been placed after *G. brevipalpis*. These four species may reasonably be regarded as hygrophilic members of the genus.

G. pallidipes has been placed in the mesophilic category; it has a very wide distribution, but in the drier parts of its range it is invariably associated with the semi-deciduous vegetation of minor drainage lines. The same may be said of *G. morsitans*, but where it overlaps with *G. pallidipes* in the central parts of its range it is associated with thicket elements of the general woodland rather than with the heavier drainage line thickets. For this reason it has been placed after *G. pallidipes* in the mesophilic category.

G. swynnertoni has been included in the xerophilic class as an inhabitant of the arid thornbush of central Tanganyika, where it is independent of the drainage line thickets. And *G. longipennis* has been placed at the end of the scale; it occurs in the Northern Frontier desert country of Kenya, in most places as the sole representative of the genus. In such areas it does appear to be dependent on drainage line vegetation, but it is likely that conditions there may be considerably more extreme than any which are to be found in lighter vegetation types in Central East Africa.

The arrangement shown in figure 3 is very similar to a sequence proposed by Buxton (1955 : 302) relating different species to the type of woodland which they generally inhabit. It differs in some points of detail from that put forward in a previous publication (Bursell, 1958), but the earlier scheme was supposed to represent the situation in respect of breeding sites, whereas the present one is concerned with the conditions of the adult environment. Granted that it is in the main a valid representation it is clear that there is no correlation between habitat and resistance to desiccation. Powers of resistance are as great in the hygrophilic group as in the xerophilic, and thus it would appear that the water balance of adult tsetse flies has been of negligible importance in the conquest of arid and semi-arid habitats; or that such adaptations as may have arisen to meet the demands of a dry environment (e.g. behavioural) have had no direct effect on resistance to desiccation as measured under present conditions.

G. morsitans and *G. swynnertoni* could be cited as apparent exceptions to the general lack of correlation; both are inhabitants of the drier types of

environment and, of the species under review, they show the greatest resistance to desiccation. Reference to figure 2 and to Table II, however, shows that they owe their high powers of resistance completely (*G. swynnertonii*) or in very large part (*G. morsitans*) to an increase in the quantity of expendable water relative to other members of the group of comparable size. The difference is in fact a reflection of a similar difference already demonstrated for the pupae of these species. In view of the very close correlation between habitat and resistance to desiccation in the pupa (Bursell, 1958) and the complete absence (except for these two species) of a correlation between habitat and resistance to desiccation in the adult, it seems very probable that the process of selection has been operative on the pupal stage—in other words that the possession of high powers of resistance by the adult tsetse flies may be no more than an incidental consequence of a similar difference in the pupa, having no selective advantage in its own right.

It must be emphasized that the views put forward relate to only one aspect of water balance, and conclusions may have to be modified in the light of further investigation. For example, it is possible that specific difference may occur in respect of the ability to regulate water loss during flight; such differences might be the cause of the observed differences in the values of the activity/loss regression coefficients shown in Table II. The coefficients tend, indeed, to be higher in the hygrophilic species; but the differences are not statistically significant and, in view of the possibility that there may be specific differences in the kind of activity exhibited under the experimental conditions, no reliance can be placed on this apparent correlation with habitat.

4. DISCUSSION

Problems analogous to the ones described in connection with size and transpiration have been encountered by workers on respiratory metabolism, and the relation between size and oxygen consumption has been extensively discussed in the literature. There appears at present to be a widespread dissatisfaction with the "surface concept" (see, for instance, Zeuthen, 1953; Ellenby and Evans, 1956; Edwards, 1958). In so far as studies of transpiration may be of relevance, the finding that changes in body surface may occur independently of changes in rate of water loss lends support to the current reaction. It is apparent that definitive statements regarding the relation between size and physiological rates cannot be attempted without a great deal more work designed to elucidate their causal background. At the present stage there is clearly little theoretical justification for the standard practice of using surface measurements as a basis for comparison between groups or species of different size. An empirically derived function of a measure chosen on the grounds of convenience would provide a more valid unit of comparison.

The importance of comparing transpiration rates at a given level of activity is illustrated by the data shown in figure 1A and B; the mean rate of loss of teneral flies is 3.2 per cent./hour and of non-teneral flies 2.4; this, however, does not reflect a difference in resistance to desiccation but is a simple consequence of the fact that the mean activity of the teneral flies is much greater than that of the fed flies; at a given level of activity there is no difference between the two groups.

A comparative study of water loss was made by Jackson (1945) using three species. The rates of water loss recorded were of the same order as those observed during the present study in spite of the fact that the humidity was 50 per cent. R.H. as compared with 0 per cent. R.H. This suggests that there must have been a great deal of activity under the conditions of Jackson's experiments ; but if the results are corrected for size (on the basis of residual dry weight) there is reasonable agreement between the two sets of observations (*cf.* Table II above). In both the rate of loss of *G. morsitans* was less than that of *G. swynnertoni* and *G. palpalis* ; the slightly higher loss of *G. swynnertoni* as compared with *G. palpalis* in Jackson's experiments may be attributed to a higher level of activity in the former species, as reflected in a somewhat higher rate of fat consumption (0.73 per cent. as compared with 0.63 per cent.).

A comparative investigation of transpiration, based on the structure and function of the spiracles, was undertaken by Geigy and Huber (1952). These authors failed to find any evidence of specific differences in the relative size of the spiracles. The factors responsible for the differences between *G. palpalis* and *G. morsitans* which have been found in the present investigation cannot therefore be ascribed to differences in spiracle size, and it seems probable that differences in the properties of the tracheal system are involved.

The results shown in figure 3 relate to the unfed fly and application of such data to the non-teneral fly must be made with some caution. It has been shown that the rate of transpiration of the fed fly is not significantly different from that of the newly emerged fly, but as yet nothing is known about the amount of water which must be lost in the processes of excretion and defaecation. However, the newly fed fly, after concentration of its blood meal by so-called "primary excretion" (Jack, 1939), carries in the undigested meal an amount of water almost equal to the total water reserves of its tissues ; and from analysis of flies captured in their natural environment it is apparent that a considerable proportion of this water becomes available for replenishment of tissue fluids (Bursell, unpublished). Thus, excretory loss apart, fed flies should be capable of supporting life for nearly twice as long as the resting teneral under the conditions of the present experiments, conditions which are far more stringent than any that even the most xerophylic of species will be subjected to in the field. It would be unprofitable to pursue this argument further in view of the uncertainties with respect to excretory loss, loss during flight and amount of daily activity in the field. But taken together with the complete lack of correlation between resistance to desiccation and habitat within the group, such considerations cast doubt on the suggestions that seasonal fluctuations in tsetse numbers are caused by variations in saturation deficit (*see*, for instance, Nash, 1933*a* and *b*).

II. THE WATER BALANCE OF NEWLY EMERGED FLIES IN THE FIELD

1. INTRODUCTION

In 1945 an experiment involving the release of a large number of *Glossina morsitans* Westw. in the habitat of *G. swynnertoni* Aust. was carried out at Shinyanga in the Lake Province of Tanganyika. Some data were published on the water content of recaptured teneral flies (Jackson, 1946), and from

these, an estimate of the water loss of such flies could be made. The rate of loss appeared to be low in comparison with those recorded in Section I of this paper, but unfortunately the design of the experiment was such as to preclude detailed analysis and no firm conclusion could be reached. The suggestion of a discrepancy seemed, however, of considerable interest, and a second release was planned with a view to investigating the matter in greater detail.

2. MATERIAL AND METHODS

Puparia of *G. morsitans* were collected near Singida in the Central Province of Tanganyika; they were brought to Shinyanga by car and were exposed in a block of natural vegetation, the habitat of *G. swynnertonii* and *G. pallidipes* Aust., on the afternoon of the same day (for a description of the area see Vicars-Harris, 1936). As it was considered unlikely that a single day's release would provide a sufficient number of recaptures for the type of analysis proposed, marking of the emerging flies was carried out on the 1st, 3rd, 4th and 5th days of the release, a different colour being used each day. The marking was done with oil paints, a small spot being placed on the thorax of flies as soon as they emerged from their puparia, while they were yet incapable of flight. The flies did not have to be handled at all, and comparison of the results obtained with the unmarked recaptures released on Day 2 has failed to reveal any effect of marking on the water loss or fat consumption of the flies.

The pupae were taken to the release site at about 12.00 hours (East African Standard Time); the rate of emergence was slow during the early afternoon and rose to a peak between 15.00 and 18.00 hours, during which interval more than 70 per cent. of the total release emerged. For determining the period during which the emerged flies had been exposed before recapture 16.30 hours was taken as the mean release time, so that in only three out of every ten recaptures will the maximum error exceed 1.5 hours.

Flies were recaptured in the afternoon between 14.00 and 19.00 hours; they were exposed immediately to potassium cyanide vapour, and, as soon as they had become immobilised, were transferred to small killing tubes lined with moist filter paper and closed by a rubber bung. Preliminary tests had shown that under these conditions there is no measurable change in weight during transport of the flies to the laboratory. The water content, fat content and residual dry weight of flies were determined according to the methods described in Section I. For comparison with the recaptured teneral a batch of flies was killed one hour after emergence from their puparia.

The results were analysed by calculating the regressions of water, fat and residual dry weight (dry weight minus fat) on size (*i.e.* thoracic surface, *cf.* Bursell, *in press* (a)) and estimating, from the regression formulae, the mean value of each of these factors at a given size. Rates of loss, both of water and of fat, have been expressed as percentages of the residual dry weight. A correction was applied to the data derived from marked flies to allow for the weight of the oil paint. Its composition after exposure to air for 22 hours or more was determined as 2 per cent. water, 55 per cent. chloroform soluble substances and 43 per cent. residual dry weight. The mean weight of marks applied to the flies was 0.07 mg., so that for marked flies 0.001 mg. was subtracted from the weight of water, 0.039 mg. from the weight of fat and 0.030 mg. from the residual dry weight.

A thermohygrograph was operated in a Stevenson screen in the shade of the tree under which the release was carried out, the readings being checked at regular intervals with reference to a mercury-in-glass thermometer and a whirling psychrometer. From the records the mean temperature and humidity to which the released teneral flies had been exposed could be calculated. Fortunately the weather was very constant during the week of the release, the mean of hourly temperatures for the four days on which recaptures were made being 27.9, 26.7, 27.2 and 27.6° C. and the mean relative humidity 29, 30, 25 and 27 per cent R.H. It was considered unnecessary to correct individual recaptures for these slight fluctuations, and the conditions to which the teneral flies were exposed has been taken as 27.5° C. and 28 per cent. R.H. The water loss and fat consumption of resting flies in the laboratory at this temperature and humidity have been estimated using methods described in Section I. A similar but much smaller release of *G. swynnertoni* was carried out using pupae collected locally.

3. RESULTS

Table IV summarises the distribution of recaptured flies; on the first day most of the flies were caught at the release site and only a small proportion was attracted to the catching party. On the second day less than half of the total recaptures was taken from the vegetation, the majority of the flies being attracted to the catching party, and on the third day the proportion taken off the vegetation had fallen to less than a quarter. These findings are in accord with previous experience (see Jackson, 1946) in so far as they suggest that activity is at a minimum on the day after emergence and rises progressively in the course of starvation.

TABLE IV.—*The numbers of teneral flies recovered after release*

	On vegetation		On catching party
	At release site	Away from release site	
<i>G. morsitans</i>			
Day 1 . .	48	19	6
Day 2 . .	10	21	50
Day 3 . .	0	4	15
<i>G. swynnertoni</i>			
Day 1 . .	3	9	7

The total number of *G. morsitans* released was 2686 and of *G. swynnertoni* 160.

Of the flies caught on vegetation, excluding those taken at the release site which happened to be under a large *Commiphora subsessilifolia* Engl., 66 per cent. were found on *Commiphora schimperi* (Berg) Engl. or *Commiphora subsessilifolia*, only 8 per cent. were taken off *Lannea humilis* (Oliv.) Engl. and the rest were distributed on seven other species of tree. This is not unlike the distribution of resting *G. swynnertoni* recorded in the same area (F. Isherwood and H. A. W. Southon, *personal communication*); in the absence of information about the density of these trees, the extent to which the distribution of the catch represents a preference on the part of the flies cannot at present be gauged.

Flies released on different days were analysed separately in respect of water, fat and residual dry weight: differences between samples reached the 5 per cent. level of significance in only two out of the 21 possible comparisons and it was considered that the data could legitimately be regarded as homogeneous. In other words marking appears to have had no effect on the water balance or activity (fat consumption) of the flies: nor have the slight day to day differences in climate been reflected in significant differences in metabolism.

TABLE V.—*The composition of released and recaptured teneral*

	N	Water (mg.)	Residual dry weight (mg.)	Fat (mg.)	Size (arbitrary units)	Time since release (hr.)
<i>I. G. morsitans males</i>						
Controls	32	16.334±0.141	5.104±0.047	1.869±0.040	7.909±0.075	.
Day 1	47	14.584±0.132	4.843±0.035	1.608±0.039	7.996±0.072	22.3
Day 2	40	13.442±0.133	4.850±0.038	1.121±0.036	8.013±0.072	47.8
Day 3	7	12.615±0.414	4.964±0.143	0.760±0.043	8.243±0.158	69.3
<i>II. G. morsitans females</i>						
Controls	35	16.477±0.107	5.162±0.042	1.835±0.049	9.389±0.090	.
Day 1	26	15.024±0.206	5.043±0.054	1.550±0.049	9.477±0.136	22.8
Day 2	41	14.031±0.173	5.097±0.039	1.168±0.038	9.293±0.106	48.0
Day 3	14	12.478±0.449	5.127±0.098	0.763±0.043	9.857±0.119	68.3
<i>III. G. swynnertoni males</i>						
Controls	21	14.506±0.122	4.457±0.048	1.395±0.035	7.457±0.085	.
Day 1	11	13.391±0.158	4.446±0.060	1.093±0.033	7.455±0.118	21.2

Comparisons of male *G. morsitans* were made at size 8.00, of female *G. morsitans* at size 9.45 and of male *G. swynnertoni* at size 7.456 and the values recorded in columns 3-5 refer to flies of these respective sizes.

A summary of the data on teneral composition is given in Table V: in no case could a significant change in regression coefficients be established so these have not been included. Column 4 shows that there is a slight decrease in residual dry weight on the first day after emergence: such a decrease can also be demonstrated with flies maintained in the laboratory, but its nature has not yet been determined. It is possible that it may be entirely accounted for by the loss of excretory products accumulated during pupal life.

The decrease in water content with time is shown in column 3 of Table V, and changes in fat content are shown in column 5. Preliminary inspection of these figures reveals an interesting anomaly, namely that for both males and females the loss of water on the first day is greater than on the second in spite of the fact that the rate of fat consumption is rising during this same period. There can be little doubt that these changes in the rate of fat consumption reflect changes in the activity of the teneral flies, and viewed in this light they are in accord with the findings presented in Table IV, that activity is slight on the first day and increases progressively in the course of teneral life. The data indicate that this increase in activity, far from causing an increase in the rate of water loss, appears on the contrary to be associated with a reduction of

transpiration. In other words it seems that the expectation based on the earlier release, that flies lose less water than they could be expected to do, is fulfilled.

In order to analyse this anomaly in greater detail it is necessary to make allowance for the amount of metabolic water produced in the course of fat consumption. Assuming complete oxidation, 1 mg. of fat should produce 1.08 mg. of water (*see* Section I, p. 216), so that a decrease from 16.334 to 14.584 mg. in the water content of male flies between day 0 and day 1 (*see* Table V, column 3) represents a water loss of $16.334 - 14.584 = 1.750$ plus the

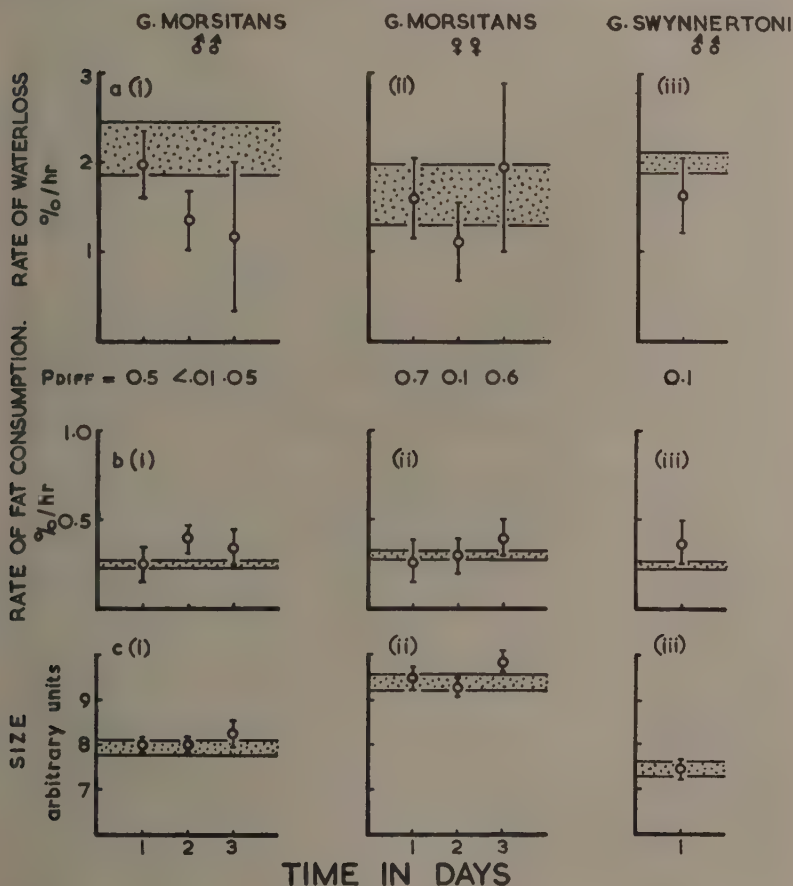


FIG. 4.—The rate of water loss (a) and of fat consumption (b) and the size of recaptured flies (c) on successive days after emergence from the puparium (*see* text).

The horizontal bands represent the resting losses and the size of controls plus and minus twice the standard error of the mean. The vertical lines through each point represent the 5 per cent. fiducial limits of the means.

The values given below (a) show the probability that the difference between recorded loss and resting loss is significant.

amount of water produced by combustion of fat during the corresponding period, namely $(1.869-1.608) \times 1.08 = 0.279$ (Table V, column 5). Thus the total water loss is $1.750 + 0.279 = 2.029$, which for a period of 21.3 hours and a residual dry weight of 4.843 represents a rate of loss of 1.97 per cent./hour. This value, and others similarly derived, have been plotted against time in figure 4.

The results show that there is a substantial decrease in the rate of water loss of males on the second day of teneral life (fig. 4*a*(i)); and that this decrease occurs in spite of a significant increase in the fat consumption (fig. 4*b*(i)), reflecting an increase in activity. The females also show a decrease in water loss associated with an increase in activity (fig. 4*a*(ii) and *b*(ii)), but in this case neither of the changes reaches the 5 per cent. level of significance.

Tables IV and V and figure 4 include some data relating to *G. swynnertoni*; it was felt that results obtained with *G. morsitans* at Shinyanga would be open to objection on the grounds that the flies were exposed in an unnatural habitat. To discover whether similar effects could be obtained with the native species, *G. swynnertoni* were released from pupae in the way described for *G. morsitans* and the composition of the recaptured flies compared with that of newly emerged tenerals to get an estimate of the water loss and fat consumption. A very limited number of pupae were available and in order to get a sufficiently large number of recaptures the experiment had to be extended over a period of about two weeks. It was carried out in September when the mean temperature was 24.6° C. and the mean humidity 36 per cent., so the values are not directly comparable with those obtained for *G. morsitans*. Only the recaptures of males on the day after release were sufficient for analysis of composition and the results depend for their significance on comparison with laboratory work (see below).

For comparison with results obtained in the field the resting water loss of male and female *G. morsitans* was determined at 27.5° C. and 27 per cent. R.H., and for male *G. swynnertoni* at 24.6° C. and 36 per cent. R.H. using the methods described in Section I. A correction was applied for the slight gain in weight occasioned by the combustion of fat, namely 0.08 mg. per mg. of fat consumed. The estimates of the rate of water loss of resting flies obtained in this way have been included in figure 4, being represented as independent of time in accordance with previous findings (see Section I, p. 212). With this basis for comparison the water loss of flies under natural conditions may be discussed in greater detail.

The fat consumption of male *G. morsitans* during the first day after emergence is very near to the resting rate as estimated from determinations of oxygen consumption (also included in figure 4), and it may be assumed that nearly the whole of the first day is spent at rest. The rate of water loss is slightly below that estimated for resting flies in the laboratory, but the difference is not significant. On the second day the fat consumption is significantly increased, denoting that some of the time is spent in activity, presumably flight. Activity causes a substantial increase in the rate of water loss under laboratory conditions in the male as well as in the female (fig. 5), yet the rate of water loss during the second day is reduced far below the resting rate, the difference between the two being highly significant. On the third day activity decreases somewhat and water loss is further depressed below the resting rate.

The situation as regards female *G. morsitans* on the first two days is the same; on the third day the females show a further access of activity and the water loss is raised to a level just above that of the resting rate, although the difference is not significant.

The fat consumption of *G. swynnertoni* on day 1 suggests that some degree of activity is exhibited within 24 hours of emergence. This is in accord with the data shown in Table IV, the proportion of flies caught on the party being

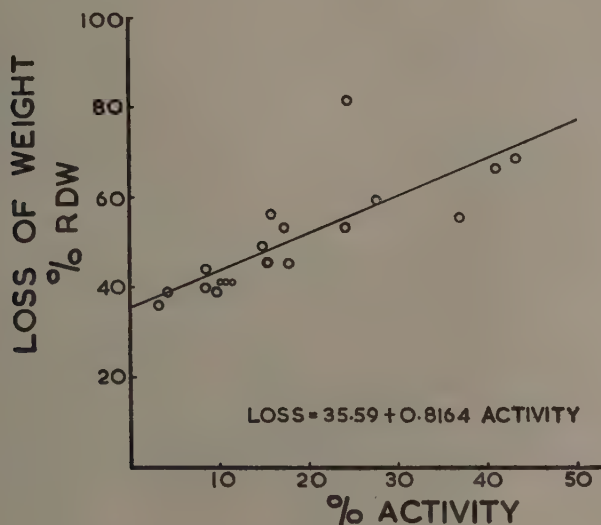


FIG. 5.—The effect of activity on the water loss of *G. morsitans* females at 27.5° C. and 28 per cent. R.H.

greatly in excess of the corresponding figure for *G. morsitans* (37 per cent. as opposed to 8 per cent.). In spite of this, the water loss is well below that which characterises the resting fly, although the difference does not quite reach the 5 per cent. level of significance.

Confining our attention for the moment to the results obtained on the second day of release it is clear that the rate of water loss as recorded by present methods is well below expectation. It is inconceivable that activity should lead to anything but an increase in the rate of transpiration, and the extra loss has not only been made good, but is in some way over-compensated so that the mean loss for the period as a whole is less than the resting value. It is necessary to enquire into the possible causes of this phenomenon.

The first possibility which must be considered is that the results may be explicable in terms of a bias in the sampling. There are two ways in which the recaptured flies may fail to provide a fair sample of the released population :—

- (1) The behaviour of flies may depend on the state of reserves, and if such behaviour affects the availability of flies to the catching party a selection may result.

(2) The composition of the population available for recapture may have been altered so that even a fair sample of the available population at the time of recapture is no longer a fair sample of the released population. This might be brought about, in the earlier stages of the experiment, by flies with smaller reserves feeding sooner than others and thus passing out of the teneral category, and in the later stages by flies with smaller initial reserves being the first to die.

That selection based on changes in behaviour may occur is shown in Table IV, where the proportionate recapture of active flies is seen to increase progressively with time, and it was necessary to inquire whether this aspect of behaviour could be correlated with a difference in the state of reserves. Day 2 is the only one for which a number of recaptures sufficient for analysis is available in both categories. The result of such an analysis is set out in Table VI. It is seen that the fat content of the resting sample of both males and females tends to be greater, although the difference does not reach the 5 per cent. level of significance. In other words behaviour does appear to be influenced by the

TABLE VI.—*A comparison between flies caught resting and flies taken on the catching party on the second day after release (G. morsitans)*

	N	Water (mg.)	Diff.	t	Fat (mg.)	Diff.	t	Size (arbitrary units)	Diff.	t
<i>I. Males</i>										
Resting .	19	13.281±0.237	.	.	1.188±0.060	.	.	8.058±0.091	.	.
Active .	18	13.604±0.162	—0.323	1.1	1.066±0.035	0.122	1.7	7.933±0.131	0.125	0.8
<i>II. Females</i>										
Resting .	12	14.024±0.408	.	.	1.273±0.074	.	.	9.358±0.170	.	.
Active .	29	14.036±0.187	—0.012	0.0	1.152±0.043	0.121	1.4	9.266±0.136	0.092	0.4

The water and fat contents represent those of males of size 8.00 and females of size 9.45.

state of reserves and the question arises whether this difference is likely to lead to a bias in the sample, and if so in what way the results are likely to be affected. The answer will clearly depend on the relative ease with which resting and active flies may be recovered. If there is no difference between the categories in this respect then the increase in the proportion of active flies recaptured will be a simple reflection of an increase in the proportion of active flies in the population and no bias will be introduced. But the fact that active flies, by pursuit of the catchers, become directly available to them suggests that their recovery may in fact be easier. Selection exercised during sampling would then favour the active part of the population and the sample would become over-weighted with flies containing smaller reserves. A bias of this kind would give an estimate of water loss in excess of that which characterises the released population, and would thus reduce the discrepancy between values obtained in the field and in the laboratory. Clearly the occurrence of this discrepancy could not be accounted for in these terms.

The fact that the distinction between resting and active flies appears to be reflected in a difference in fat content suggests that sampling errors discussed in section (2) above may be of importance; for it is reasonable to suppose that

the difference in fat content may in fact be the cause of the difference in behaviour, regarding activity in this case as an instance of food seeking. On this interpretation the evidence suggests that there is a tendency for flies with smaller reserves to feed sooner than others, and the question arises whether this tendency would be likely to alter the composition of the remaining teneral population sufficiently to affect conclusions based on an analysis of the recaptured sample.

In this connection it may be emphasised in the first place that the process of selection appears to operate in relation to fat reserves only; there is no difference between the water contents of active and resting samples (see Table VI), and this fact alone would tend to reduce any errors introduced by the bias of the sample in so far as fat consumption contributes only between 30 and 40 per cent. to the estimated rates of loss.

Secondly, a test for the occurrence of selection in the combined samples on which the data of figure 4 are based may be derived by the following consideration:— Given the regressions of fat and of residual dry weight on size, the relation between the former variables can be determined (the relevant formulae are $\text{Fat} = 0.6125X - 3.031$ and $\text{Residual dry weight} = 0.7233X - 0.682$ from which $\text{Fat} = 0.8468\text{RDW} - 2.453$, where $X = \text{Size}$). With this relation it is possible to compare the fat content of flies of different size and such a comparison shows that large flies possess proportionately more fat than small ones. For instance the fat content of a fly which has a residual dry weight of 4.4 mg. is 22.4 per cent. of the total dry weight, while that of a fly which has a residual dry weight of 5.8 mg. is 29.8 per cent. Given in addition that the resting rate of fat consumption is 0.27 per cent./hour it can be calculated that the small fly will in two days have lost more than half while the large one will have lost less than a third of its fat reserves.

This difference between large and small flies does not apply to water reserves; these are proportionately equal over the whole range of sizes, and the percentage depletion of reserves is also equal (p. 216).

Since large flies have the advantage over smaller ones in respect of fat reserves, it follows that any selection based on these reserves should be reflected in a corresponding selection for size. That this argument is valid is suggested by the data in Table VI, which shows that in a case where a selection based on fat reserves can be demonstrated a similar selection for size is also evident (see last columns). It would seem legitimate to draw the converse conclusion and maintain that where no selection for size can be demonstrated there will be no reason to assume that results will be significantly affected by a selection based on food reserves.

In figure 4c are shown the mean sizes of successive samples compared with those of the released populations (control). It is seen that for all the data relating to days 1 and 2 the mean size of recaptured samples is extremely close to that of the controls, and such departures as do occur may be ascribed to random errors of sampling. The samples recaptured on day 3 are of greater mean size than the original released populations, the difference being statistically significant only for females.

The fact that selection occurs only on the third day suggests that the selective process may differ from the one which, during early teneral life, appears to operate on the basis of feeding propensity. A study of the individual

regressions does in fact show that in males, for instance, the fat content of flies smaller than 7.1 has reached the limit of tolerance (*i.e.* 4.2 per cent. of the total dry weight); such flies make up about 10 per cent. of the original population. It seems that at this late stage in teneral life selection is based on the death of flies which started with small food reserves, a selective process more inexorable than one based on the *tendency* for such flies to feed and in this way to be eliminated from the teneral category. It is of interest to note that the water content of such small flies, as estimated from the relevant regressions, would have been well above the critical level.

In conclusion it may be said that selection of flies with relatively extensive food reserves in the sample of recaptures, based on their smaller propensity to feed, is too slight in the combined samples of days 1 and 2 to be reflected in deviations of mean size from that characteristic of the released population. Such selection may therefore be presumed to be too slight to affect conclusions drawn from differences in composition between such samples and the control population. On the third day selection through exhaustion of food reserves and death appears to be at work and data obtained at this time should be accepted with caution. In actual fact the magnitude of errors caused by such selection can be calculated, as it will be a simple function of the difference in initial food reserves corresponding to the difference in size between the control and the selected populations. For males this error in the calculated rate of water loss amounts to no more than 0.13 per cent./hour, which is negligible compared with the observed discrepancy (*see* fig. 4).

A second possibility which might account for the abnormally low rates of water loss as calculated above is that flies have access to sources of water other than blood. The suggestion has in fact been put forward in recent years that tsetse flies may ingest flower nectar (Evens, 1954) but the evidence in favour of this or any source of water other than blood is illusory. The question has been discussed by Buxton (1955), who concludes that the possibility may legitimately be dismissed although it cannot be absolutely excluded.

A third possible reason for the low rates of loss recorded is partial feeding of the recaptured flies; but this is not very likely—flies were carefully inspected at the time of capture for traces of blood; as a further precaution the residual dry weight of each fly was plotted against size, and results for individuals whose points lay clearly outside the normal scatter of the plots were excluded from the calculations. Deviations from the regression line seldom exceeded 0.2 mg., and since a full blood meal has a residual dry weight of about 6.0 mg. it is clear that very small feeds would have been capable of detection. Admittedly the possibility that a few flies which had taken a very small meal have been incorporated in the samples cannot be rigidly excluded; but that such flies constitute at the most exceptions is shown by the constancy of the residual dry weight in successive samples (*see* Table V).

It seems then that selection and other artifacts may be dismissed and that the low rates of water loss shown by teneral flies in the field must be accepted as a real phenomenon. There remains the question of its interpretation.

It must first be emphasised that the phenomenon does not rely for its demonstration on a comparison between laboratory maintained and naturally occurring flies. If it did it might be objected that laboratory flies were in some way abnormal and for that reason lost water at higher rates than wild flies

under comparable conditions, although indeed there is no evidence of such abnormality. But a comparison between the composition of wild flies on day 1 and day 2 suffices to show the existence of a comparable anomaly, namely that resting flies on day 1 lose more than partially active flies on day 2 in spite of the fact that conditions are constant or practically so.

The most reasonable interpretation of the results as a whole would seem to be that the flies are making use of some microclimate so that they are not in fact exposed to the conditions which obtain generally in the habitat. During the first day of teneral life the use which is made of such microclimates would appear to be slight, except perhaps in the case of *G. swynnertoni* (see fig. 4a(iii)).⁴ On the second day, however, it would seem that a very considerable proportion of time would have to be spent in microclimates of high relative humidity; for the observed loss is less than that shown by flies at rest in the laboratory in spite of the fact that some activity had occurred in the field, to judge from the increased fat consumption. Some data are available on the oxygen consumption and water loss of tsetse flies in flight, and from these it is possible to get some idea of the magnitude of the humidity difference necessary to produce the observed effect. Thus at 27.5° C. and 30 per cent. R.H. the oxygen consumption increases by a factor of 22 and the water loss by a factor of 6 during flight (Bursell, *in press* (b)). Using these values it can be calculated that the increased fat consumption of males between day 1 and day 2 would represent a flight period of 35 minutes and during this interval 7.3 per cent. of water would be lost. The total loss between day 1 and day 2 would thus be 7.3 per cent. plus a resting loss of 53.4 per cent., giving a total of 60.7 per cent. The actual loss recorded in the field was 32.4 per cent. In order to attain this low level of loss it would thus be necessary for the fly to spend half of its time under conditions of negligible water loss, that is at 100 per cent. R.H.; alternatively it would have to spend all its time at a humidity about 50 per cent. higher than the ambient; or suitable combinations of duration and humidity between these two extremes would achieve the observed reduction. These estimates are evidently subject to considerable error, but they do give some idea of the sort of effect one would have to look for, and, above all, they indicate that an explanation of the observed effect in terms of microclimate may, at least theoretically, be within the bounds of possibility.

Turning now to the likelihood of this interpretation in the light of field experience, it may be said that the evidence does not entitle us to dismiss it out of hand. On the positive side there is the association of the resting flies with species of *Commiphora* and *Lannea*; these are distinguished amongst other dominant species of the thorn bush in having extremely thin and often green bark, suggesting the possibility that photosynthesis, and hence transpiration, may be characteristic of the surfaces of their branches. To what extent this might affect the microclimate at the level of the spiracles of a resting fly has not yet been determined. It may be mentioned that a kinetic reaction has been demonstrated which would tend to confine flies to a microclimate of this kind, where it is available (Bursell, 1957a).

⁴ In view of the fact that the deviations from the resting rate are in the direction expected on the basis of previous work, the confirmation of which was the primary aim of the investigation, this would seem to constitute a case where probabilities may legitimately be halved.

Secondly, I have been permitted to quote a relevant discovery, made by members of this Department (Mr. F. Isherwood and Mr. H. A. W. Southon, *personal communication*), namely that during a very limited interval of time just before dark resting flies of *G. swynnertoni* have been observed to leave their normal daytime perches on the underside of branches; by means of artificial light they have subsequently been discovered resting on the upper surface of leaves, a site which they seem seldom to employ in daylight. The converse move takes place in the early morning soon after dawn. Here again the occurrence of a microclimate at the surface of transpiring leaves seems a possibility.

On the negative side there is the fact that we are by no means certain that the observed resting sites of the tsetse fly (*e.g.* Nash, 1952; Nash and Davey, 1950; Isherwood, 1957) are the only ones, nor indeed that they are the most widely used. So far it has not been possible to get an estimate of the time spent by different portions of the population in known resting sites, and until this can be done an element of doubt must remain associated with observations on this aspect of tsetse ecology.

Thus the field data, although they cannot be said to offer any material confirmation of the hypothesis put forward to account for low rates of transpiration, are at least not at variance with such an explanation. A final solution of the problem must await further refinements of ecological and laboratory techniques.

In addition to confirming the existence of the anomaly suggested by the 1945 release, the data shown in Table V are of interest in connection with the supposed causes of death in natural populations of tsetse fly. This has been a much debated question and it is doubtful if it could even be said that a consensus of opinion exists in the matter. It has, however, been the custom in much of the field work on tsetse flies to relate seasonal fluctuations in apparent density to similar fluctuations in saturation deficit on the assumption that there is some causal connection between high evaporating powers and low fly densities (*e.g.* Nash, 1933*b*; Jack, 1942; Jackson, 1948). Evidence has been presented above which suggests that a proportion of the population actually dies of starvation before the flies have a chance to take their first meal. Reference to Table V will show that the state of food reserves in the survivors is precarious, amounting to no more than 13 per cent. of their total dry weight; with 4.2 per cent. as the lower limit this means that at this time the flies have used more than two-thirds of their available reserves. The water reserves of the 3 day-old teneral, on the other hand, have been relatively little depleted; their water content is about 71 per cent. which, with a critical level of 64.5 per cent. (Section I, Table III), means that little more than half of the reserves has been lost. It must be emphasised that conditions in the experimental area were by no means natural in respect of host availability since catching parties and release parties were operating in the area for a substantial proportion of the daylight hours. But although this disturbance might have produced the appearance of starvation as an artifact, it is not likely to have affected the relative rates of utilisation of fat and water reserves. So the results suggest that in spite of the extremely arid conditions which obtained at the time of the experiment, flies were in no danger of death by desiccation.

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IV. SUMMARY

Water Balance in Flies at rest

1. A method is described for estimating the water loss of resting tsetse flies. Corrections for size were made on the basis of residual dry weight, both fresh weight and thoracic surface having been found to be unsatisfactory measures of size.

2. The powers of resistance to desiccation of different species were determined by consideration of their resting loss in relation to the quantity of expendable water reserves.

3. For the species studied there was no correlation between resistance to desiccation and habitat. It is tentatively concluded that the water balance of the adult tsetse has been no obstacle to the invasion of semi-arid and arid habitats.

Water Balance in Flies in the field

1. Releases were made of newly emerged *G. morsitans* and *G. swynnertoni* in the natural habitat of *G. swynnertoni*.

2. The recaptured flies were analysed in respect of water, fat and residual dry weight, and by comparison with a control sample killed within an hour of emergence the rate of water loss and of fat consumption was determined for successive days of teneral life.

3. The rates of water loss occurring in the field were compared with estimates of the resting loss determined in the laboratory under the same conditions of mean temperature and humidity.

4. Except for a single batch of recaptures the flies recovered from the field had lost water at rates lower than those shown by resting flies in the laboratory, in spite of the fact that the rate of fat consumption of the released flies showed that they had been active for some of the time before recapture.

5. It is suggested that the anomaly may be accounted for on the assumption that flies make use of microclimates of high relative humidity, and this possibility is considered in the light of field observations.

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